PICRO-CARMINE AND ALUM-CARMINE AS COUNTER STAINS.

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The following paper embodies the results of experiments with picro-carmine and alum-carmine* as counter stains, as developed incidentally during the year in the histologic laboratory at Cornell University.

The excellence of picro-carmine was first noticed last November, in staining developing bone which had been decalcified. Picro-fuchsin† was being regularly used as a counter stain with hematoxylin. Merely for the experiment picro-carmine was used on one section and left nearly two hours. Much to our surprise and pleasure we found that, instead of our section being ruined, we had secured an excellent differentiation. This was not the first attempt with picro-carmine, but always before the time had been short, from two to fifteen minutes.

The advantage of the stain over picro-fuchsin is noticeable in the superiority of differentiation secured as illustrated in the slides presented at the meeting of Microscopical Society.

The embryonal cartilage cells are better marked by the hematoxylin and picro-carmine, for the alkaline picro-carmine does not fade the hematoxylin as does the acid picro-fuchsin.

It is particularly in the zone of calcifying cartilage that this superior differentiation is noticed. The vertically arranged rows of cartilage cells have lost their horizontal septa, but the

*For literature see Lee's Vade Mecum and the most recent publications on the subject by P. Mayer, Ueber Picro-carmin, Zeitschrift für wissenschaftliche Mikroskopie. Vol. XIV, pt. I, p. 18.

†See Freeborn, Trans. N. Y. Path. Soc., 1893, p. 73. Also Studies from the Dept. of Path. of the College of Physicians and Surgeons, Columbia University, N. Y., 1894-5.

vertical septa are pronounced and project into the primary marrow cavity as irregular trabeculae of calcified cartilage. The osteoblasts have enveloped these trabeculae with a covering of true bone and at the same time the cartilaginous trabeculae within are being absorbed and true bone substituted.

This true bone, with the picro-carmine, has taken a red which is brilliant in comparison with picro-fuchsin; and the gradually diminishing and disappearing cartilage which, with picro-fuchsin, has taken a stain not distinguishable from that of the cells of the true bone is, with picro-carmine, beautifully differentiated by a clear pronounced blue, showing the alkalinity of the picro-carmine.

This tendency on the part of picro-carmine to bring out the hematoxylin as a blue, while the acid picro-fuchsin fades it, is very noticeable in the tonsil of dog which was next submitted. In the mucous cells near this gland the nuclei, removed as far as possible from the lumen, are brought out with unequalled clearness. The structure of the blood vessels is also brought out with great distinctness, and the differentiation throughout is very marked.

Quite as striking a contrast between picro-carmine and picro-fuchsin is noticed in a section of the pyloric stomach of a kitten. The stain with picro-carmine is not only more differential, but the unstriped muscle of the stomach and blood vessels is brought out much better by the picro-carmine.

During the summer picro-carmine was tried with good results on sections of the fallopian tube of a mare. It has been used with greatest success on tissues which present a mucous surface, and while these successes have been noted, an equal number of failures were encountered, so no claim is made for picro-carmine as a "pan" stain. It seems particularly unsuited for tissues that stain with difficulty.

Ranvier's picro-carmine was used in most of these experiments, but Bizzozero's was used with equal success. Mayer's recent formula was used in the histological laboratory at Cornell last year with results quite as good as those from Ranvier's.

In the summary, then, we find picro-carmine, in the cases noted, gives, with hematoxylin, a more differential stain than picro-fuchsin, and shows the characteristic alkaline reaction with hematoxylin, bringing out the hematoxylin as a beautiful sharp blue, while the acid picro-fuchsin tends to fade it. Two hours is, in general, the best time for picro-carmine. There is no danger of overstaining.

ALUM-CARMINE.

During the summer it was my privilege to prepare some slides of liver of guinea pig to show Anthrax bacilli. The bacilli were readily found, and, at the request of Dr. Moore, pathologist and bacteriologist of the New York State Veterinary College, I attempted to get a contrast stain and finally succeeded with alum-carmine. I had tried picro-carmine without success. In fact I have never been able to secure a good stain with picro-carmine on liver. By experiment I found that one hour and fifty minutes with alum-carmine gave the best results. The crystal-violet with which the bacilli were stained, and which is washed out much or entirely by the alcohols and clearer, must be sufficiently intense to permit of thorough dehydration and clearing and yet leave a distinct stain. One and one-half minutes will suffice if care is taken not to leave longer than is necessary in alcohol.

By this stain the nuclei and the cell body are clearly differentiated and the alum-carmine forms a very good contrast stain with the crystal-violet. The simplicity of the method commends it to us. It is suggested that with methylene blue a still greater contrast may be secured.

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ADDENDUM.

Since writing the above my attention has been called to the fact that Stöhr in his text book of Histology (p. 156, Second Ed., translated by Dr. Billstein) directs that developing bone be stained with hematoxylin and then with picro-carmine.

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